



A New Whey Cheese Analogue Made from Whey Protein Concentrate and Vegetable Fat with 15% Olive Oil

Evangelia Zoidou, Ioanna Andreadaki, Theophilos Massouras and Stelios Kaminarides*

Department of Food Science and Human Nutrition, Agricultural University of Athens, Greece

***Corresponding author:** *Stelios Kaminarides, Department of Food Science and Human Nutrition, Laboratory of Dairy Science and Technology, Agricultural University of Athens, Iera Odos 75, Votanikos, 11855 Athens, Greece, E-mail: skamin@aua.gr*

Abstract

In this study a whey cheese analogue was made using as basic ingredients whey protein concentrate 65 and vegetable fat with 15% olive oil. The product was enriched with thyme leaves, film packed and then subjected to heat treatment. The overall characteristics of the product were investigated during 25 days storage at 4°C and compared with those of traditional whey cheese. The whey cheese analogue had a lower microflora than traditional whey cheese, a smaller percentage of saturated and a greater percentage of mono- and poly-saturated fatty acids and remained organoleptically and texturally acceptable after 25 days storage at 4°C whereas traditional whey cheese deteriorated. The whey cheese analogue was characterized as a new whey cheese easy to produce with improved health enhancing properties, acceptable textural characteristics, rich aroma profile and extended shelf-life.

Keywords

Whey cheese analogue, Whey protein concentrate, Vegetable fat, Whey cheese

Introduction

Imitation cheeses or cheese analogues are cheese-like products in which milk fat, milk protein or both are partially or wholly replaced by non milk-based components to produce a specific cheese variety. They are manufactured by blending various edible fats/oils, proteins, other ingredients and water into a smooth homogenous blend with the aid of heat, mechanical shear and emulsifiers [1]. The market of analogues has grown recently due to their simplicity of production, and the substitution of lacteal ingredients by cheaper vegetable ones, factors that allow for a reduction in product manufacturing costs [2].

The production of whey cheeses is based on the denaturation and coagulation of the water soluble milk proteins present in the whey when it is heated at temperatures above 85°C [3]. Of all Greek whey cheeses, Myzithra is the one that is produced in the largest quantity throughout the country. It is a soft cheese, usually made from the whey of hard or semi-hard cheeses or derived after Feta cheese production from either ewe or a mixture of ewe and goat milk. Myzithra is more a dietary product compared with other Greek traditional cheeses, containing 15-19% fat and a moisture content that is not greater than 70% [4]. Due to the high moisture content, low concentration of salt and pH close to 6, Myzithra, is susceptible to microbial spoilage and

consequently has a limited shelf-life [5,6]. Whey Protein Concentrates (WPCs) are whey protein powder preparations which contain 35-80% total protein and variable quantities of moisture, lactose, ash, fat, K, Cl, Ca, P, Na, Mg and non protein nitrogen, while the total microorganism count is influenced by the following processing steps: pasteurization, ultrafiltration, spray-drying and packaging. WPCs are widespread due to their functionality and nutritional value, as they contain high concentrations of bioactive proteins, essential amino acids, e.g. branched chain amino acids (leucine, isoleucine, and valine) and sulphur amino acids (methionine, cysteine) and they have antioxidant properties [7].

Fat consumption has been shown to be associated with an increased risk of heart and artery disease [8]. Milk fat contains over 70% saturated acyl groups and of these, laurate, myristate and palmitate are considered particularly atherogenic [9]. The amount of fat intake is considered equally important to the balance of saturated to unsaturated fatty acids, so that diets abundant in mono and polyunsaturated fats are considered healthy. Vegetable oils are a good source of healthy unsaturated fats and are generally cholesterol-free. Substitution of milk fat by vegetable fat in dairy products is an option for obtaining products with a healthier saturated/unsaturated fat balance [10]. However, the incorporation of vegetable oils causes modifications in cheese microstructure and textural behavior [11,12].

Taking into consideration the importance of utilizing WPCs and the demand for healthy products that are consistently available in the market all year round, the aim of this study was to prepare a whey cheese analogue using as basic ingredients whey protein concentrate (WPC) and a vegetable fat blend with 15% olive oil. Olive oil has many beneficial properties (contains vitamins E and A acting antioxidants contributing to longevity, prevents the formation of blood clots in the heart arteries, prevents fat accumulation in the liver, etc). In order to improve flavour, the product was enriched with thyme leaves and to extend its shelf life it was film packed before being subjected to heat treatment. The product was stored at 4°C for 25 days and analyzed for physicochemical, microbiological, rheological and organoleptic characteristics in comparison with traditional whey cheese.

Materials and Methods

Production of whey cheese analogue

Whey cheese analogue was prepared so as to adjust the fat in dry

matter to about 50% and moisture content to about 67%, as in control traditional whey cheese, using the following formula: (1) 61.3% distilled water, (2) 17.2% Whey Protein Concentrate 65% (WPC-65) from the Greek “Epirus Protein Company” containing 65% serum proteins, 23% lactose, 3.8% fat, 3.3% ash, calcium 3.6 g/kg, phosphate 3.3 g/kg, sodium 2.7 g/kg, potassium 6.6 g/kg, magnesium 0.8 g/kg, (3) 1.7% skim milk powder containing 1% fat, 51% lactose, 27% caseins, 6.6% serum proteins and 8.5% ash, (4) 19.8% vegetable fat containing 15% olive oil, (5) 0.4% emulsifier/stabilizer (E/S) powder (Cremodan SE 334 VEG type, Danisco). The WPC and milk powder were mixed thoroughly in the water under stirring and gradually heating to 65°C. At 65°C, vegetable fat, then E/S and finally fresh thyme leaves were added to the mix and agitated to produce a thick, smooth, homogenous mixture. The mixture was placed in cylindrical polyethylene film packages of 200 mL, tied firmly with string at the ends and heated in a water bath under stirring at 90°C for 30 min. Then it was cooled under running water to room temperature and maintained refrigerated at 4°C for 25 days.

Production of traditional whey cheese

Traditional whey cheese was produced according to the following procedure described by Anifantakis [13]: whey derived from sheep and caprine milk in a ratio 70/30 following the production of Halloumi cheese [14] was collected, filtered to remove any existing curd grain, weighed and poured into a circular cheese vat. The whey was gradually heated under continuous stirring up to 85°C. When small curd particles of whey proteins appeared, the temperature was increased to 90°C, while stirring was reduced and finally stopped when a very thin layer of coagulum formed on the surface of the whey. The curd was cooked at 90°C for 30 min, then transferred gradually by a perforated ladle into moulds of cheese cloth and hung from a pole in a well-ventilated room at 20°C for 3 h to drain. Afterwards, the whey cheese was wrapped in paper and maintained refrigerated at 4°C for 25 days.

Cheese sampling

Five replicates of each type of cheese were produced. The cheeses were analyzed 1 day after production and after 25 days of storage at 4°C. All analyses were performed in triplicate. All reagents and chemicals were of analytical grade.

Microbiological analysis

Cheeses were examined for their total bacteria microflora, yeasts and moulds and coliforms. 10 g of the cheese sample were blended with 90 mL of 2% (w/v) sterilized sodium citrate (Merck, Darmstadt, Germany) for 2 min in a Stomacher Lab Blender 400 (Seward Medical, London, UK) and then subjected to serial dilutions using 0.1% (w/v) Ringer solution (Oxoid, Hampshire, England).

The following microbiological tests were performed: total aerobic bacteria on PCA Agar (Difco, Michigan, USA) at 30°C for 72h [15], yeasts and moulds on YGC-agar (Merck, Darmstadt, Germany) at 25°C for 5 days [16], coliforms on Violet Red Bile Agar (Oxoid, Hampshire, England) at 37°C for 24 h [17]. All the counts were expressed as colony forming units per gram of cheese (cfu g⁻¹).

Physicochemical analysis

Cheeses samples were analyzed for total proteins [18], total solids [19], fat [20], ash [21], acidity [22], minerals [23] and lactose [24]. The pH was measured by a pH meter (632, Metrohm, Germany).

Analysis of volatile compounds

The volatile compounds profile of cheese samples was measured 1 day after production using SPME GC/MS analysis. Cheese samples (4 g) were homogenized with 2 mL of saturated Na₂SO₄ aqueous solution and 100 µL of an internal standard aqueous solution containing 0.77 g L⁻¹ cyclohexanone (Sigma-Aldrich Quvmica, Alcobendas, Spain). Aliquots (3 g) of the homogenates were placed in to 22 mL vials sealed with PTFE/silicone septa (Supelco, Bellefonte, PA) through which the SPME syringe needle (bearing a 50/30µm DVB/CAR/PDMS fiber

Supelco, Bellefonte, PA, USA) was introduced. The samples were stirred continuously on a stir plate revolving at 750 rpm. Fiber was exposed to the headspace above the sample for 30 min at 65°C. The absorbed volatiles were then analyzed by GCMS (Shimadzu GC-17 A, MS QP5050 and capillary column HP-INNOWax 60 m, 0.25 mm i.d, 0.25 µm film thickness, J&W Scientific, Agilen Technologies). Interface, quadruple and ion source temperatures were 250, 280 and 230°C respectively. The oven temperature was set at 45°C for 5 min, increased to 150°C at a rate of 5°C min⁻¹, then raised at 7°C min⁻¹ to 220°C and held at 250°C for 20 min. Helium was used as carrier gas at a flow rate of 1.0 mL min⁻¹. Electron impact ionization of MS was used at a voltage of 70 eV. The mass range was *m/z* 40-500. Identification was effected by comparison with standards and using the NIST (National Institute of Standards and Technology, United States) spectrum library. The volatile compounds were quantified by dividing the peak areas of the compounds of interest by the peak area of the IS, multiplying this ratio by the initial concentration of the IS (expressed as ppm). The peak areas were measured from the full scan chromatograph using total ion current (TIC).

Fatty acids analysis

For fatty acids analysis, lipid extraction was performed [25] and fatty acids were then methylated by base-catalyzed methanolysis of milk fat [26]. The fatty acid methyl esters (FAME) were analyzed by GCMS (Shimadzu chromatograph GC-17A, equipped with flame ionization detector (FID) and capillary column SP-2340 30 m, 0.25 mm id, 0.2 µm film thickness, Supelco Bellefonte, PA). Injection and detector temperatures were 250°C and 270°C respectively. The injection volume was 1 µL (split 1:50). The oven temperature was set at 45°C for 5 min after injection, increased to 150°C at a rate of 5°C min⁻¹, held there for 5 min and then raised at 7°C min⁻¹ to 220°C and held there for 20 min. Helium was used as carrier gas at a flow rate of 1.0 mL min⁻¹. The FAME identification was performed by comparing with standards (FAME Mix Supelco, Sigma-Aldrich) and quantification was performed using Dionnex Chromeleon software.

Textural evaluations

The textural profile analysis (TPA) of the cheeses was assessed with a Shimadzu testing instrument, model AGS-500 NG (Shimadzu Corporation, Kyoto, Japan) equipped with a 5-kg load cell, as described by Kaminarides and Stachtiaris [27]. The following six textural parameters were calculated: Hardness (N), defined as the peak force (H) during the first compression cycle (first bite), is the force necessary to attain a given deformation. Cohesiveness (N mm), defined as the ratio of the positive area under the curve during the second compression to that during the first compression. Adhesiveness (N mm), defined as the negative force area for the first bite, is the work necessary to overcome the attractive forces between the surfaces of the cheese and the plunger with which the cheese comes into contact. Elasticity (mm), defined as the ratio of the base line of the positive curve during the second compression to that during the first compression, is the height that the cheese recovers during the time that elapses between the end of the first and the start of the second bite. Gumminess (N), which is the product of hardness X cohesiveness, is the energy required to disintegrate a cheese to a state ready for swallowing. Chewiness (N), which is the product of gumminess X elasticity is the energy required to masticate a cheese to a state ready for swallowing.

Organoleptic evaluation

Cheese samples were graded by a taste panel of the Dairy Laboratory of the Agricultural University of Athens. Panel members evaluated cheese for appearance, body and texture, and flavour (odor and taste) using a 10-point scale, with 1 being the worst and 10 the best quality. More importance was given to flavour and to body and texture than to appearance of the cheese as advised by IDF standard [28], by multiplying their scores by 5 and 4, respectively. The total score was obtained by adding the scores of the three attributes. An excellent cheese obtained a total score of 100.

Table 1: Microbial counts (log₁₀ cfu/g) of the two types of whey cheese 1 day after production and after 25 day's storage^a.

Microbial group	Traditional whey cheese		Whey cheese analogue	
	Day 1	Day 25	Day 1	Day 25
Total bacteria	3.61 ^a ± 0.43	8.94 ^c ± 0.20	1.77 ^a ± 0.48	6.37 ^b ± 0.2
Coliforms	< 1 ^a	7.60 ^b ± 0.27	< 1 ^{a**}	< 1 ^a
Yeasts	2.04 ^b ± 0.53	6.28 ^c ± 0.57	< 1 ^a	< 1 ^a

^aValues are means ± standard error of mean. Different letters (a,b,c) in the same row are indicative of a significant statistical difference ($P < 0.05$) between the values.

^{**}Not found in the sample dilution 10⁻¹.

Table 2: Physicochemical characteristics of the two types of whey cheese 1 day after production^a.

Physicochemical characteristics	Traditional whey cheese	Whey cheese analogue
Fat %	18.39 ^a ± 2.04	16.27 ^a ± 0.49
Moisture %	66.86 ^a ± 1.58	67.34 ^a ± 0.65
Ash %	1.01 ^b ± 0.06	0.81 ^a ± 0.02
Total protein %	11.93 ^a ± 0.47	12.01 ^a ± 0.16
pH	6.25 ^a ± 0.07	6.20 ^a ± 0.24
Lactose %	3.17 ^a ± 0.28	4.66 ^b ± 0.05
Ca (mg/100 g cheese)	246.71 ^b ± 25.97	90.04 ^a ± 9.60
Mg (mg/100 g cheese)	18.90 ^a ± 1.60	17.57 ^a ± 2.11
K (mg/100 g cheese)	77.89 ^a ± 6.33	132.71 ^b ± 15.66
Na (mg/100 g cheese)	14.83 ^a ± 0.52	64.23 ^b ± 6.24

^aValues are means ± standard error of mean. Different letters (a,b) in the same row are indicative of a significant statistical difference ($P < 0.05$) between the values.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Software Statgraphics Plus for Windows v. 5.2 (Manugistics, Inc., Rockville, MO, USA) to test the effect of different cheese type on cheese parameters. The effect of storage time on the cheese characteristics was studied using the Statistical program (StatSoft, Inc, 2010).

Results and Discussion

Enumeration of microorganisms

The microbiological results 1 day after production and after 25 days of cold storage are shown on [table 1](#). Fresh whey cheeses are susceptible to microbial spoilage owing to contamination and sporulation after heat processing of the whey had taken place [29]. As shown in [table 1](#), 1 day of cold storage traditional whey cheese had a total bacterial count (TBC) of 3.61 log cfu g⁻¹ and yeasts 2.04 log cfu g⁻¹, while coliforms were not detectable in sample dilution 10⁻¹ due to the thermal processing of the whey and adequate sanitary conditions during the production of this cheese. Bacteria counted as TBC were thermotolerant, psychrotrophic bacteria, the spores of which are activated immediately after the heat treatment of milk by forming vegetative forms [30]. After 25 days cold storage in traditional whey cheese, the total bacteria increased to 8.94 log cfu/g, while yeasts increased to 6.28 log cfu/g due to the growing of these microorganisms. Also, the coliforms were 7.60 log cfu g⁻¹. Their presence is possibly attributed to the entry of environmental contaminants during cheese preservation as the traditional whey cheese was wrapped in paper after its production. The microbial content of the traditional whey cheese was in accordance with previously reported data for Myzithra [31] and whey cheese, Anthotyro [5].

On the other hand whey cheese analogue on day 1 had less total bacteria (1.77 log cfu g⁻¹) and also not detectable coliforms on day 25, due to the packaging of the whey blend in polyethylene bag before heating. So, by the end of cold storage the traditional whey cheese deteriorated as microorganisms were found in higher counts than those of whey cheese analogue and this could be attributed to the different method for packaging for both cheeses. On the contrary, the new whey cheese analogue has a lower microflora when fresh and better sustainability for 25 days.

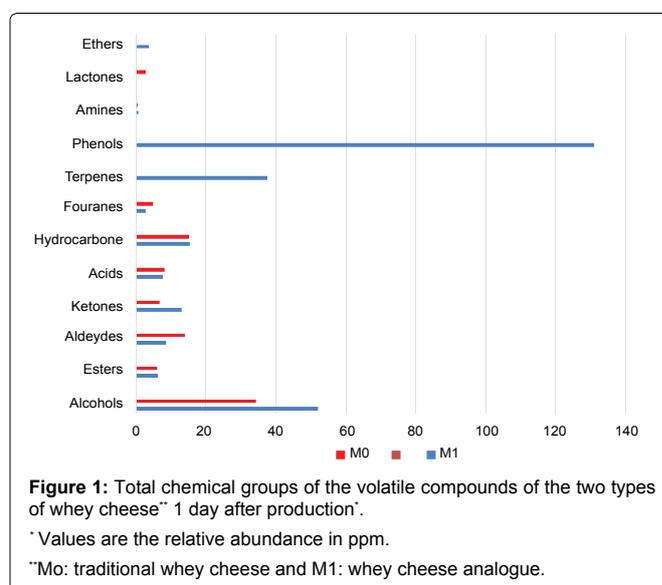


Figure 1: Total chemical groups of the volatile compounds of the two types of whey cheese^a 1 day after production^a.

^aValues are the relative abundance in ppm.

^{**}M0: traditional whey cheese and M1: whey cheese analogue.

Physicochemical characteristics

The results of the physicochemical analysis of the 1-day-old cheeses are given in [table 2](#). There were no significant differences ($P > 0.05$) in protein, fat and moisture content between the different cheeses. Both of them had a mean moisture content of 67%. The differences ($P < 0.05$) in ash content found between traditional whey cheese and the whey cheese analogue may be attributed to differences in the mineral content of the mixture. Calcium was higher in traditional whey cheese, but potassium and sodium content were present in greater amounts in the whey cheese analogue due to the composition of the added WPC. The higher mean lactose content (4.66%) in the whey cheese analogue can be attributed to the composition of the added WPC and milk powder.

No significant differences ($P > 0.05$) in the physicochemical characteristics were found between day 1 and day 25, apart from the pH values which decreased significantly due to microbial growth ($P < 0.05$). 25-day-old traditional whey cheese had a lower pH (5.17) than the corresponding whey cheese analogue (5.72) due to its higher bacteria population.

Volatile compounds

The volatile compounds included alcohols, esters, aldehydes, acids, ketones, terpenes, fouranes, hydrocarbons, phenols, lactones, ethers and amines, and their relative abundance is presented in [figure 1](#). Most of them have been previously reported for whey cheese Myzithra [32]. Alcohols were the most abundant compounds in traditional whey cheese accounting for 37.07%, followed by hydrocarbons, aldehydes, acids and ketones. In the whey cheese analogue, phenols thymol and carvacrol, which are the principal volatiles of thyme leaves [33], accounted for 47.02% of the total VOC of this cheese, giving it a characteristic flavor, followed by alcohols (18.66%) and terpenes (13.50%). The high level of alcohols could be attributed to their production from many metabolic pathways such as lactose metabolism, methyl ketone reduction, amino acid metabolism and the degradation of linoleic and linolenic acids [34].

Fatty acids profile

The percentages of fatty acids of cheeses on day 1 are shown in [table 3](#) and classified as Saturated Fatty acids (SFA), Mono-unsaturated Fatty acids (MUFA) and Poly-unsaturated Fatty acids (PUFA). It is known that the SFA are potentially harmful to humans, especially with respect to coronary heart disease [8]. The percentage of SFA in the whey cheese analogue was lower compared to the traditional whey cheese (50% and 68.5% respectively), with palmitic, stearic and myristic acids dominating. In addition, the percentage of low molecular fatty acids C4-C10 in the whey cheese analogue was just 0.28% as

milk fat was replaced by vegetable oil, while in traditional whey cheese it was 17.97%. The new cheese analogue had a higher percentage of MUFA and PUFA (34% and 15.5% respectively), containing oleic acid, linoleic (LA) and dihomo- γ -linolenic acid (DGLA) in the highest amounts. The above omega-6 fatty acids are well-known for their beneficial properties in human health, as they reduce total and LDL cholesterol levels and protect against heart diseases [35].

Textural assessment

Instrumental texture profile analysis indicated differences between the two types of whey cheese in texture attributes, except elasticity (Table 4). Thus on day 1 the whey cheese analogue was softer, less cohesive, gummier, adhesive and chewier than traditional whey cheese. Milk fat in traditional whey cheese gave a more compact and continuous protein matrix. In contrast, when vegetable oil was used in the whey cheese analogue, a looser, less

dense and therefore weaker and more disrupted protein matrix was formed. This was due to the low melting point of vegetable fat and oleic acid resulting in the presence of more numerous droplets, in comparison with the higher melting point of milk-fat, which resulted in fewer globules [11]. Consequently, the whey cheese analogue was characterized by reduced hardness, chewiness and cohesiveness [36]. The lower lever of the above parameters could also be attributed to the lower Ca content. The lower adhesiveness of the whey cheese analogue combined with its high spreading ability could be associated with its high olive oil content. The results for adhesiveness and cohesiveness in traditional whey cheese were in agreement with those from previous studies [32]. With respect to elasticity, both cheeses exhibited similar elasticity values, although this property is largely unimportant for whey cheese.

After 25 days of storage, no significant ($P > 0.05$) decrease in all the rheological parameters of traditional whey cheese was observed due to proteolysis, whereas in whey cheese analogues most of these parameters increased substantially. Proteolysis disrupts the structural integrity of the protein matrix, leading to reduced firmness cohesiveness and chewiness [37].

Table 3: Percentages of fatty acids (% fatty acids / of total fat) of the two types of whey cheese 1 day after production.

Fatty acid		Traditional whey cheese	Whey cheese analogue
	SFA*		
Butiric acid	C ₄	4.17	0.00
Caproic acid	C ₆	2.99	0.01
Caprylic acid	C ₈	3.12	0.10
Capric acid	C ₁₀	7.69	0.17
Lauric acid	C ₁₂	3.59	0.92
Myristic acid	C ₁₄	10.73	1.29
Pentadecanoic acid	C ₁₅	0.82	0
Palmitic acid	C ₁₆	28.06	38.72
Nacre acid	C ₁₇	0.4	0.07
Stearic acid	C ₁₈	6.97	3.46
Arachidonic acid	C ₂₀	0.00	0.62
Eicosanoic acid	C ₂₁	0.00	4.95
	MUFA*		
Myristoleic acid	C _{14:1}	0.42	0.03
Palmitoleic acid	C _{16:1}	1.06	0.17
Oleic acid	C _{18:1 (cis-9)}	25.42	34.04
	PUFA*		
α -Linoleic acid	C _{18:2 (cis-9, cis-12)}	2.69	11.63
α -Linolenic acid	C _{18:3 (cis-9, cis-12, cis-15)}	0.15	0.09
Dihomo- γ -linolenic acid	C _{20:3}	0.00	3.73
CLA**	C _{18:2 (cis-9, trans-11)}	1.73	0
Total		100	100

*SFA: Saturated Fatty acids, MUFA: Mono-unsaturated Fatty acids, PUFA: Poly-unsaturated Fatty acids

**CLA: Conjugated linoleic acid

Organoleptic evaluation

The results of the organoleptic evaluation are presented in table 5. Whey cheese analogues containing vegetable fat could be sliced similarly to traditional whey cheese but was softer, had a yellowish color because of the vegetable fat used and a characteristic thyme flavor due to the added thyme leaves. Although the scores awarded by the taste panel were lower for the whey cheese analogues than for traditional whey cheese on day 1 for all the attributes evaluated ($P < 0.05$), the total score of the former was nevertheless quite high (78-91%).

There were no significant ($P > 0.05$) differences in any of the organoleptic characteristics of the whey cheese analogues after 25 days of storage. On the contrary, traditional whey cheese on day 25 received a lower overall score ($P < 0.05$) than on day 1, since it had developed an acid taste and substandard texture due to the proteolytic and lipolytic activities of aerobic and psychrotrophic bacteria, which are usually responsible for the production of many metabolites leading to the development of off-flavours in foods [30].

Conclusion

A whey cheese analogue was successfully and easily manufactured using as basic ingredients whey protein concentrate WPC 65 and vegetable fat with 15% olive oil in fat. The final product resembled the traditional whey cheese in physicochemical composition but had increased unsaturated fats, a richer aromatic profile, lower microflora and better keeping quality during cold storage.

Table 4: Rheological characteristics of the two types of whey cheese 1 day after production and after 25 day's storage*.

Rheological characteristics	Traditional whey cheese		Whey cheese analogue	
	Day 1	Day 25	Day 1	Day 25
Hardness (N)	4.60 ^b \pm 0.21	4.23 ^b \pm 0.29	1.39 ^a \pm 0.15	1.87 ^a \pm 0.19
Elasticity (mm)	1.13 ^a \pm 0.10	1.00 ^a \pm 0.001	1.01 ^a \pm 0.01	1.05 ^a \pm 0.05
Cohesiveness (N.mm)	0.45 ^b \pm 0.02	0.43 ^b \pm 0.02	0.38 ^a \pm 0.01	0.43 ^b \pm 0.02
Adhesiveness (N.mm)	14.93 ^c \pm 2.26	12.63 ^c \pm 2.13	5.24 ^a \pm 0.67	8.6 ^b \pm 1.23
Gumminess (N.mm)	2.05 ^b \pm 0.08	1.89 ^b \pm 0.15	0.53 ^a \pm 0.06	0.8 ^a \pm 0.13
Chewiness (N.mm)	2.29 ^d \pm 0.11	1.89 ^c \pm 0.15	0.54 ^a \pm 0.06	0.8 ^b \pm 0.05

*Values are means \pm standard error of mean. Different letters (a,b,c,d) in the same row are indicative of a significant statistical difference ($P < 0.05$) between the values.

Table 5: Organoleptic evaluation of the two types of whey cheese 1 day after production and after 25 day's storage*.

Organoleptic characteristics	Traditional whey cheese		Whey cheese analogue	
	Day 1	Day 25	Day 1	Day 25
Texture (0 - 40)	37.05 ^a \pm 0.13	34.72 ^b \pm 0.10	30.52 ^a \pm 0.21	31.20 ^a \pm 1.18
Taste (0 - 50)	44.80 ^c \pm 0.20	28.33 ^a \pm 2.73	40.16 ^b \pm 0.37	41.04 ^b \pm 1.20
Color (0 - 10)	9.58 ^c \pm 0.10	8.78 ^b \pm 0.15	7.78 ^a \pm 0.47	8.01 ^a \pm 0.13
Total (0 - 100)	91.43 ^c \pm 1.56	71.83 ^a \pm 2.63	78.46 ^b \pm 2.97	80.24 ^b \pm 2.21

*Values are means \pm standard error of mean. Different letters (a,b,c) in the same row are indicative of a significant statistical difference ($P < 0.05$) between the values.

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